THE INTERACTION BETWEEN THE SYNAPSES OF A SINGLE MOTOR FIBER

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INTRODUCTION

A favorable preparation for the study of properties of simple synapses is provided by the giant fiber system of the crayfish, *Cambarus*. Previous anatomical and physiological studies (Johnson, 1924, 1926; Wiersma, 1947) have shown that certain motor fibers in the third roots of the abdominal ganglia of this animal have at least four synapses arranged linearly along the length of the neurite. These synapses, shown diagrammatically in Fig. 1, are: (1) with the heterolateral medial giant fiber of the abdominal nerve cord; (2) with the homolateral first sensory root of the same abdominal ganglion; (3) with the homolateral medial giant fiber of the abdominal cord; and (4) with the homolateral lateral giant fiber of the abdominal cord. The two lateral giant fibers of the central nervous system are interconnected at frequent intervals throughout their course, so that the third root may be activated by stimulation of either lateral giant fiber. There is no actual synapse, however, between a third root giant fiber and the heterolateral lateral giant fiber (Fig. 1).

The synapses between the central giant fibers and the motor giant fibers of the third roots consist merely of places where these fibers touch, without discernible anatomical differentiation in the walls of the synapting fibers. The exact position of the synapse between the third root fibers and entering afferent fibers of the homolateral first root (synapse 1–3, Fig. 1) is uncertain. Physiological evidence, however, tends to place it in the position shown in the diagram. None of the synapses mentioned will conduct antidromically.

In the fresh condition all these synapses transmit impulses from pre- to postsynaptic fiber in a 1:1 fashion. However, when the synapses are fatigued by higher frequency stimulation, a 2:1 or higher ratio may become established between pre- and postsynaptic elements. This higher ratio may be maintained for long periods of time by proper adjustment of stimulus ferquency. If the frequency of stimulation of the presynaptic element is sufficiently high, the

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synapse will block, or "fatigue," completely. A short rest will usually re-establish the original 1:1 relationship (Wiersma and Schallek, 1948).

It has been found further that, when a synapse is blocked by fatigue, summation of presynaptic impulses may occur; *i.e.*, a postsynaptic impulse will result if the interval between two presynaptic impulses is optimum. In these instances it has been established that the summating presynaptic impulses do not need to be delivered through the same presynaptic fiber, but that any combination of presynaptic elements may be used. Different combinations of presynaptic fibers yield, of course, different time relations of the periods in which summation is possible. From such experiments it may be concluded that an increase in excitability occurs along the length of the third root giant fiber when any one of its synapses is activated, even though no propagated postsynaptic impulse may occur. There is thus evidence for one kind of mutual interaction between the synapses of the third root giant fibers.

In the present paper still another aspect of mutual interaction between synapses is investigated, in this case the depressant action of one synapse on another. Fatigue of one synapse may sometimes prevent other synapses from functioning, even though these other synapses have not themselves been previously stimulated. The conditions under which such blocking action occurs are here clarified.

During the course of the present experiments a functional connection, not previously noted, between the two medial giant fibers was discovered. The characteristics of this connection will be briefly described, since they form a contribution to the normal physiology of the giant fiber system and since they may be a serious source of error in interpretation of the type of experiments reported here.

Materials and Methods

All experiments were performed on the crayfish *Cambarus clarkii*. By techniques previously described (Wiersma, 1947) the lateral and medial giant fibers were isolated and prepared for stimulation in the circumoesophageal commissures. The abdominal cord, with its ganglia and rootlets, was carefully freed from adjacent tissues. Action potentials were led from the third abdominal roots through a Grass P3 amplifier to a Du Mont 279 oscilloscope, the sweep of which was triggered from the stimulating circuit.

The synapses were studied in pairs. Two presynaptic pathways at a time were adjusted on micromanipulated platinum electrodes. Through one set of electrodes one pathway was continuously stimulated at a frequency of 20 shocks per second until the pre- post-synaptic junction of that pathway became fatigued and blocked. Through the other set of electrodes the second pre- post-synaptic pathway was tested during and after stimulation of the first pathway. In this way the effectiveness of transmission at the various synapses could be tested. After 5 minutes' rest, at the end of which time the synapses had returned to their normal state (Wiersma and

Schallek, 1948), the experiment was repeated with the order of stimulating and testing of the two pathways reversed. Whenever possible, this procedure was repeated several times.

As previously stated, the synapses tested were, as shown in Fig. 1: (1) between the heterolateral medial giant fiber and the third root (HEM-3, Fig. 1); (2) between the homolateral first root and the third root (1–3, Fig. 1); (3) between the homolateral medial giant fiber and the third root (HOM-3, Fig. 1); (4) between the homolateral lateral giant fiber and the third root (LG-3, Fig. 1). Since the lateral giant fibers are interconnected, it makes no difference which of the two is stimulated. However,

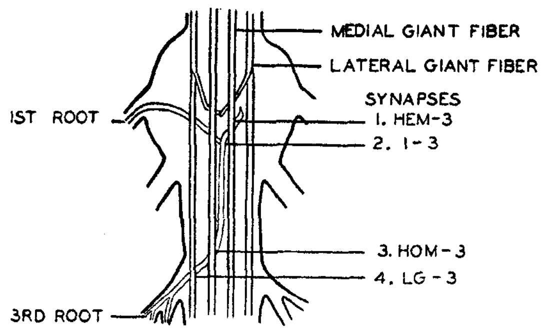

FIG. 1. Diagrammatic representation of the arrangement of giant fibers in an abdominal ganglion of the crayfish. The pair of medial giant fibers and the pair of lateral (interconnected) giant fibers are shown synapting with the giant fibers passing out the third motor root of an abdominal ganglion. The exact anatomical location of the synapse between the first root and the third root (1–3) is not known. The synapses in order are: HEM-3, between the heterolateral medial giant fiber and the third root; 1–3, between entering afferent fibers in the homolateral first root of the same ganglion and the third root; HOM-3, between the homolateral medial giant fiber and the third root; LG-3, between the homolateral lateral giant fiber and the third root.

there is no direct connection between the heterolateral lateral giant fiber and the third root fiber (Wiersma, 1949).

The recording electrodes were arranged in such a way that the action potentials of the giant fibers in the cord were visualized on the same trace as the action potentials from the third roots (for method see Wiersma, 1947). Records were usually taken from the third root of the third abdominal ganglion. Throughout the course of the experiments the preparations were maintained in crayfish physiological solution (van Harreveld, 1936).

RESULTS

In almost all cases it was found that a block established at any given synapse consistently prevented transmission from any more centrally located synapse (Table I). For example, it was usually possible to prevent transmission from the three more centrally located synapses (HEM-3, 1–3, HOM-3, Fig. 1) by fatiguing the synapse between the lateral giant fiber and the third root (LG-3, Fig. 1).

Activity at peripheral synapses (*e.g.* LG-3) often blocked transmission at more centrally located synapses considerably before the peripheral synapse itself became fatigued. Moreover, this block at the more centrally located synapse may occur in even less time than it would take to block that same central synapse by stimulation of its own presynaptic fiber. For example, a HOM-3 synapse which normally fatigued at the end of 41 seconds' stimulation of its own presynaptic fiber, failed to respond to a presynaptic impulse in that fiber after the LG-3 synapse had been stimulated for only 12 seconds. The LG-3 synapse itself took 150 seconds to become blocked.

TABLE I

Mutual Influence of Fatigue of One Synapse on Another

Synapse combination	No. of cases	Peripheral on central	Central on peripheral	
			Strong	Weak
LG and Hom.	9	8	1	1
LG and Hem.	4	4	0	0
Hom and Hem.	3	3	0	0
LG and 1st root.	3	2	0	1
Hom and 1st root.	6	5	0	3
Hem and 1st root.	3	3	1	2
	28	25	2	7

In only three out of twenty-eight cases did stimulation of a peripheral synapse to the point of fatigue fail to have a blocking action on a more centrally located synapse (Table I). In at least one of these cases there is good reason to believe that the failure of peripheral synapses to block central ones was due to an anatomical anomaly of the central giant fibers.

In the light of our experience it would appear that anatomical variations are relatively frequent in the central nervous system of the crayfish. We met for example, an extreme instance in which both medial giant fibers ran with one lateral giant fiber in the right circumoesophageal commissure. The left commissure contained only a single lateral giant fiber. Histological sections showed that an atypical arrangement extended throughout the entire cord. This unusual arrangement of giant fibers resulted in an abnormal order in which synapses were made with the third root fibers throughout the abdominal cord.

The influence of central synapses on more peripheral ones was, in general, slight. In most cases no influence whatever was present. In other cases there was a "weak" effect, and in only a few cases was the effect as marked as that of peripheral synapses on central synapses. In Table I there are two columns in which the influence of central on peripheral synapses is tabulated. In the column marked "strong" are those experiments in which activation of a central synapse completely suppressed activity of a more peripheral one before becoming blocked itself. In the column marked "weak" are listed those cases in which activity at a more peripheral synapse was reduced before or at the time of fatigue of a more centrally located synapse. In many instances in which fatigue of a central synapse failed to produce any change in the activity of a more peripheral synapse, stimulation of the presynaptic fiber of the central synapse was continued for 5 minutes after the synapse itself had become blocked. In none of these cases was any subsequent effect on a more peripheral synapse noted.

As is evident from Table I, the combinations of synapses which most frequently showed an effect of central synapse on peripheral synapse were those in which the first root–third root synapse (1–3, Fig. 1) was one of the pair. Out of twelve cases in which the first root–third root synapse was involved, in seven cases its stimulation had a noticeable effect on a more peripheral synapse. However, in these same twelve cases the first root–third root synapse was completely blocked in ten cases by stimulation of a more peripheral synapse. It may be noted also that the influence of the first root–third root synapse is more frequent and more marked on its nearest neighbor (HEM-3) than on the other two synapses (HOM-3 and LG-3, Fig. 1).

Connection between the Two Medial Giant Fibers

An incidental observation made during the course of these experiments led to an investigation of a functional connection, not previously noted, between the two medial giant fibers. It was observed at times that stimulation of an isolated medial giant fiber in one circumoesophageal commissure gave rise in the abdominal nerve cord to a double action potential, the second spike following the first by approximately 1 millisecond. By leading off from the isolated medial giant fiber in the opposite, non-stimulated, commissure it was shown that this fiber was responsible for the extra spike. A considerable number of preparations were then investigated, and the same result was obtained in more than half the cases. In several instances this functional interconnection between the medial giant fibers was very transient. By making appropriate cuts rostral and caudal to the point of stimulation it could be demonstrated that the cross-over took place in the brain. The characteristics of this connection in the brain could then be compared with the cross-over between the lateral giant fibers (see Fig. 1) previously reported (Wiersma, 1947). In such experiments the caudal part of the abdominal cord was cut in order to remove the junction between the lateral giant fibers in the telson, which is very resistant to fatigue. Both giant fibers of one commissure were then placed on a pair of stimulating electrodes, while both giant fibers of the other commissure were placed on the recording electrodes. With this arrangement two action potentials are obtained, as shown in Fig. 2. The first spike represents the action potential in the medial giant fiber which has crossed over in the brain; the second spike represents the action potential of the lateral giant fiber which has descended to the thorax, crossed over, and ascended to the recording

FIG. 2

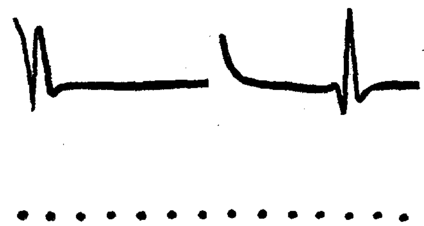

FIG. 3

FIG. 2. The isolated medial and lateral giant fibers are both stimulated simultaneously in the left circumoesophageal commissure. Recording is from the isolated medial and lateral giant fibers in the opposite circumoesophageal commissure. The first spike following the shock artifact represents the action potential of the medial giant fiber which has ascended to the brain, crossed to the opposite side, and descended to the recording electrodes. The second spike represents the action potential of the lateral giant fiber which has descended to the thoracic cord, crossed to the opposite side, and ascended to the recording electrodes. Because one of the recording electrodes was essentially indifferent, the impulses appear to be traveling in the same direction. Time in milliseconds.

FIG. 3. Stimulation with a double shock of both medial and lateral giant fibers in the left circumoesophageal commissure in a partially fatigued preparation. Recording from both medial and lateral giant fibers in the opposite commissure. On the first shock the medial giant fiber transmits an impulse through the synapse in the brain; the junction between the lateral giant fibers does not transmit an impulse. On the second shock the junction between the medial giant fibers, apparently not facilitated, does not transmit an impulse; the junction between the lateral giant fibers, however, characteristically transmits a summated postsynaptic impulse.

electrodes. By stimulating with double shocks approximately 5 milliseconds apart, further information concerning the nature of the junction between the giant fibers was obtained. As was expected, the junction between the lateral giant fibers showed characteristics similar to those of the synapses of the third roots. For example, double presynaptic impulses will summate to produce a postsynaptic impulse at a previously fatigued junction. With regard to the junction between the medial giant fibers, however, when skipping of impulses occurred as a result of fatigue, double shocks produced a postsynaptic response with no greater frequency than single presynaptic shocks alone. Fig. 3 shows this situation in which the first of a pair of shocks produces a spike through the junction between the medial giant fibers in the brain; the junction between the lateral giant fibers, since it is fatigued, does not transmit the first impulse. The second shock summates to transmit an impulse at the junction between the lateral giant fibers, but no such summation is seen with regard to the medial giant fibers, and hence no medial fiber spike is seen following the second shock. The results of such experiments may mean that the connection between the medial giant fibers does not represent a true synapse but rather a sort of fusion of the fibers, with a relatively long refractory period at the point of juncture. An apparently comparable connection in the brain exists between the single pair of central giant fibers of *Callianassa* (Turner, 1950).

It should be emphasized that we observed this functional connection between the medial giant fibers readily only in preparations made especially for this purpose; *i.e.*, those in which the medial giant fibers were quickly and carefully dissected. Impairment of conduction by electrode pressure and interference with circulation to the brain are probable reasons why this cross-connection of the medial giant fibers is rarely noticed in "normal" preparations.

DISCUSSION

It has been pointed out previously that at least three separate processes can be distinguished in the transmission of impulses across the synapses of the giant fibers of the third motor roots. These are: prolongation of the refractory period of the root fiber, depression of excitability of the root fiber, and facilitation of transmission through its synapses. It was shown that facilitation may spread from one synapse to another along the third root without the aid of a conducted action potential. Depression at a synapse may be maintained for an indefinite period by stimulation of its own presynaptic fiber. From the present results it is evident that depression can also be produced and maintained at other synapses, especially when they are located central to the synapse being stimulated.

Central or peripheral location appears to have little influence in the mutual facilitation of synapses. A much more important consideration in these cases is the actual distance between synapses. Neighboring synapses facilitate each other much more readily than do those which are separated by one or two intervening synapses. With regard to the depressant effect reported here, however, no such relation with distance has been found. In this case the relative position of the synapses, central or peripheral, appears to be the main factor. This may be considered a polar effect. This difference between facilitation and depression of synapses was striking and unexpected, and many experiments were carried out to test its validity. For this purpose, preparations in which blocking times of peripheral and central synapses were markedly different were further tested with double shocks to determine mutual facilitation of these same synapses. With both synapses of the pair completely blocked, central synapses facilitated peripheral ones to the same degree as peripheral synapses facilitated central ones.

Our experiments seem to accord with the conclusion that the direction of propagation of impulses along the third roots plays an important role in genesis of block at the various synapses. In only a relatively few instances did impulses arising at central synapses have marked depressant effects on more peripheral ones. Antidromic (peripheral to central) impulses frequently have, as shown, a marked depressant effect on synapses, and this effect may be even greater than the depressant effect set up at that same synapse by its own presynaptic fiber.

The notion might be considered that the block produced at a central synapse by stimulation of a more peripheral one is simply a case in which an impulse arising from a central synapse fails to pass a fatigued point on the third root. This idea is inadequate to account for the facts that (1) summation occurs readily even when both central and peripheral synapses are completely blocked and (2) the central synapse frequently becomes blocked long before the peripheral synapse fatigues.

Further experiments will be necessary to elucidate these differences between facilitation and depression. At present it may be concluded that evidence has been presented for the existence of a depressant effect of some synapses on others, and that this effect spreads much more readily centrally than peripherally.

SUMMARY

It has been shown that stimulation of synapses of the giant motor fibers of the third roots of *Cambarus clarkii* can block transmission at other synapses located on the same fiber. Peripherally located synapses block most synapses which are more centrally located. The reverse is true in a small number of cases. Possible reasons for this difference are discussed.

It was further found that the two medial giant fibers in fresh, carefully dissected, preparations show a functional connection in the brain. It is probable that, under natural conditions, both medial giant fibers are always active at the same time.

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